

12 **EUROPEAN PATENT APPLICATION**

21 Application number: 90106397.4

51 Int. Cl.⁵: **A61K 9/107, A61K 9/127**

22 Date of filing: 04.04.90

30 Priority: 05.04.89 IL 89856
27.02.90 IL 93558

43 Date of publication of application:
10.10.90 Bulletin 90/41

54 Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

71 Applicant: YISSUM RESEARCH DEVELOPMENT
COMPANY OF THE HEBREW UNIVERSITY OF
JERUSALEM
46 Jabotinsky Street
Jerusalem, 92 182(IL)

72 Inventor: Simon, Benita
33/3 Haarazim Strasse, Mevasseret Zion
Jerusalem 90805(IL)
Inventor: Menashe, Levy
15/5 Haarazim Strasse, Beith Hakerem
Jerusalem 96181(IL)

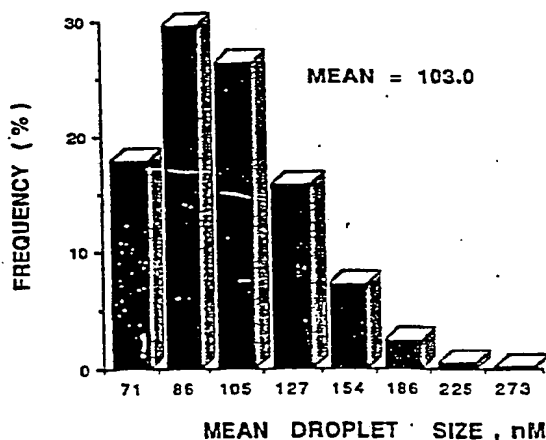
74 Representative: Vossius & Partner
Siebertstrasse 4 P.O. Box 86 07 67
D-8000 München 86(DE)

56 **Medicinal emulsions.**

57 A pharmaceutical composition being an oil in water type emulsion which comprises an effective amount of a lipophilic drug is provided. The composition comprises a synergistic combination consisting of about 3-50% of an oily carrier consisting of MCT oil optionally in combination with vegetable oil, about 0.05%-20% of a phospholipid, about 0.03-10% of a non-ionic surfactant, and about 0.05-5% of an ionic surfactant selected from the group consisting of bile-duct surface active agent, cholic acid and their derivatives.

Also provided is a novel method for preparing said composition.

DROPLETS SIZE DISTRIBUTION OF PLAIN EMULSION



EP 0 391 369 A2

DROPLETS SIZE DISTRIBUTION OF AMPHOTERICIN EMULSION

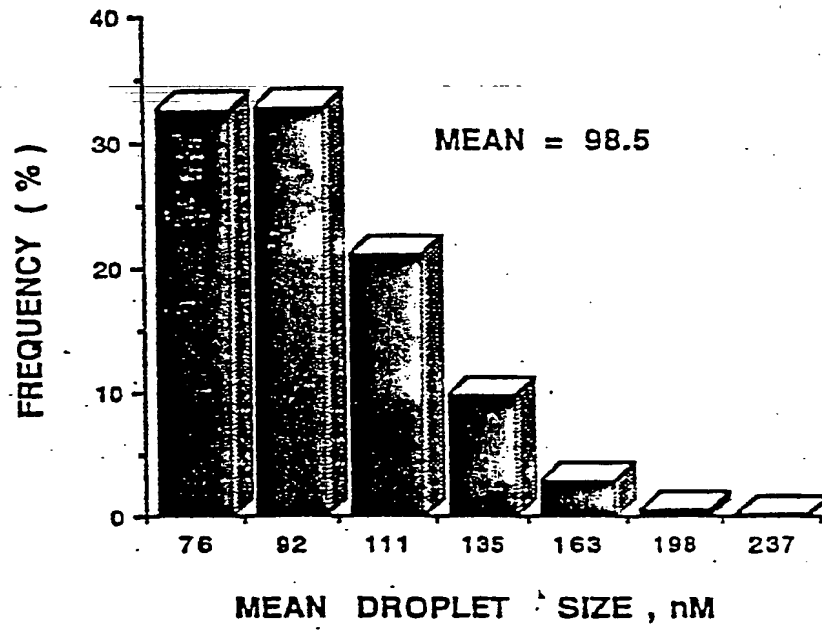


FIG.3

Medicinal Emulsions

The present invention concerns pharmaceutical compositions of hydrophobic drugs being in the form of an oil-in-water emulsion. The pharmaceutical compositions provided by the present invention show an outstanding long term stability and, in addition, in various forms of administration they also have sustained release characteristics. These pharmaceutical compositions when comprising a heat resistant drug are also remarkably stable to sterilisation by autoclaving.

Relevant prior art to the subject of the present invention may be found in the following publications:

1) U.S. Patent No. 3,172,816.

2) EP-A2-214561.

3) EP-A3-215313.

4) Benita, S., Friedman, D. and Weinstock, M. (1986), International Journal of Pharmaceutics, 30: 47-55.

5) Singh, M. and Ravin, J. (1986), J. Parenteral Sci. Technol. 37: 34-41.

6) Von Dardel, O., Mebius, C. and Mossberg, T. (1976), Acta Anaesth. Scand. 20:221-224.

The utilization of pharmaceutical compositions in the form of emulsions of drugs having low aqueous solubility is well known in the art. However, as a rule such compositions are of low stability due to fast phase separation between the oil and the water phases, which is further enhanced by the hydrophobic drugs for which the emulsions serve as carriers.

Many hydrophobic drugs are important for various medical treatments, but since they are relatively unstable and insoluble in water their most useful form of administration is by way of an oil-in-water emulsion-type composition, in which the drug is dissolved in the oil phase. This is in effect the only practical way by which such hydrophobic drugs can be administered intravenously.

In accordance with the prior art stability of compositions of this type was unsatisfactory since as stated above, the hydrophobic drugs destabilize such compositions. Another drawback of prior art emulsion type compositions is that they tend to lose their stability when they are sterilized in an autoclave which is the most efficient and least costly way of sterilizing such compositions. During autoclaving the oily droplets of the emulsion coalesce and consequently creaming and/or phase separation occurs. This therefore necessitated hitherto the use of other forms of sterilization such as filtration as described, for example, in EP-A2-214661 and EP-A3-215313.

It is the object of the present invention to provide compositions of the oil-in-water type emulsions containing hydrophobic drugs, which are stable over prolonged storage and furthermore where the drug is heat resistant, are capable of being sterilized by autoclaving without a change in their properties or loss of their stability. It is a further object of the present invention to provide such compositions for parenteral, oral, ocular and topical administration of said drug.

In accordance with the invention it has surprisingly been found that oil-in-water type emulsions of a hydrophobic drug which comprises an oily carrier being a mid chain triglyceride (MCT) oil optionally in combination with vegetable oil, phospholipids, non-ionic surfactants and an ionic surfactant being a bile-duct surface active agent or a cholic acid derivative are surprisingly more stable than prior art lipophilic drug-containing emulsions both in prolonged storage and in autoclaving.

The improved stability of the compositions in accordance with the invention was found to be caused by a synergism which exists between the various ingredients, i.e. between said oily carrier, phospholipids, non ionic surfactant and said ionic surfactant.

In the following specification and claims all indication of percent (%) are by weight (w/v - weight of ingredient/volume of entire composition). All concentrations given below should be understood as standing each by itself and not accumulative.

The invention provides a pharmaceutical composition being an oil-in-water type emulsion comprising an effective amount of a hydrophobic drug, characterised in that it comprises about 3-50% of an oily carrier consisting of MCT oil optionally in combination with of vegetable oil, about 0.05-20% of a phospholipid, about 0.03-10% of a non-ionic surfactant, and about 0.05-5% of an ionic surfactant selected from the group consisting of ionic bile-duct surface active agent, cholic acid, their surface active derivatives and their salts.

An oil-in-water type emulsion generally comprises tiny oily droplets comprising the oily carrier (to be referred to hereinafter as the "oily phase") which are suspended in an aqueous solution (to be referred to hereinafter as "the aqueous phase"). The oily droplets are surrounded by a stabilising interfacial film (to be referred to hereinafter for the sake of convenience as the "intermediate phase") formed by the phospholipids, the non-ionic surfactant and the said ionic surfactants.

MCT oil has many advantages over vegetable oil, amongst which are the following: lower susceptibility

to oxidation; having a specific density of 0.94-0.95 which is higher than that of vegetable oil and which is close to that of water thus facilitating the obtaining of a stable emulsion; being less hydrophobic than vegetable oil and therefore enables achieving higher concentrations of the drug dissolved therein; having a lower viscosity which again enables obtaining a higher concentration of the oily phase in the composition
 5 - without substantially increasing its viscosity.

On the other hand, vegetable oil has the advantage over MCT oil in its lower price. Thus, although the use of MCT oil by itself as the oily carrier is generally preferred in accordance with the invention, it may at times be practical to substitute some of it with vegetable oil.

Compositions in accordance with the present invention are suitable for topical, parenteral, ocular and
 10 oral administration of lipophilic drugs. Preferably the compositions are supplied in unit dosage form. Where a composition of the present invention is to be used for parenteral administration, it must be sterile, which sterilization is preferably performed by autoclaving. The ingredients in compositions to be used for parenteral administration will have to be of injection grade and medically approved for such administration.

An injectable composition should not be too viscous. As a rule, the viscosity of an emulsion increases,
 15 with an increase of the relative proportion of the non aqueous phase namely the oily and the intermediate phases, which comprise the oily carrier, phospholipids, non-ionic surfactant, said ionic surfactant and the hydrophobic drug. It is accordingly preferred, in accordance with the present invention, that the relative proportion of the non aqueous phase in injectable composition, should not exceed about 30%. It is even more preferred, in accordance with the present invention, that the relative proportion of the non aqueous
 20 phase in injectable compositions be below about 25%.

On the other hand, topical compositions should preferably be viscous and to this end the relative proportion of the non-aqueous phase should preferably be above about 30%.

The preferred ranges of ingredients of injectable pharmaceutical compositions according to the invention are: oily carrier - about 10-20%; phospholipid - about 0.5-2%, 0.75%-2% being particularly
 25 preferred; non-ionic surfactant - about 0.5-3%; said ionic surfactants - about 0.3-10%, 0.5-2% being particularly preferred; drug - about 0.02-2%. Again, these preferred ranges are to be understood as standing each by itself and are not cumulative.

The preferred pH of the aqueous phase of the composition of the invention is about 5.0-8.5, while 6.0-8.0 being more preferred, especially for parenteral administration.

30 Examples of MCT oil to be used in the compositions of the present invention are TCR (trade name -Societe des Oleagineux, France), Miglyol 812 (trade name -Dynamit Nobel, Sweden).

Examples of vegetable oil which may be used in the compositions of the present invention are soybean oil, cotton seed oil, olive oil, sesame oil.

Examples of phospholipids which may be used in compositions according to the invention are lecithins;
 35 Epikuron or Ovothin 160 (trade name - both manufactured by Lucas Meyer, Germany) which are mixtures of mainly phosphatidylcholine and phosphatidylethanolamine from a natural source, such as purified egg yolk phospholipids (for the Ovothin series) and such as soybean oil phospholipids (for the Epikuron series); a purified phospholipid mixture; Phospholipids E-80 (trade name - manufactured by Lipoid KG, Ludwigshafen, FRG).

40 An example of a non-ionic surfactant which may be used in compositions according to the invention is Poloxamer (Pluronic F-68 BASF, Ludwigshafen, FRG), which is a copolymer of polyoxyethylene and polyoxypropylene.

Examples of said ionic surfactant to be used in compositions according to the invention are cholic acid and deoxycholic and surface active derivatives and salts thereof, which may be obtained commercially
 45 (Sigma, St. Louis, MO., U.S.A.). Preferred are cholic acid and dexoycholic acid and their sodium salts, deoxycholic acid and its sodium salt being particularly preferred.

The ionic surfactants used in accordance with the invention are anions of relatively weak acids and may thus associate with protons to become electrically neutral when the pH is reduced. Since these substances are surface active in their anionic state, the pH during preparations should be such in which the acid is
 50 dissociated into the anions and protons, i.e. the pH should have a higher value than the pKa (pKa - the pH in which half of the acid is dissociated). It was found however that after the composition had already been prepared, there was no reduction in stability even upon lowering of the pH.

The pharmaceutical compositions of the present invention preferably also comprise a preservative such as methyl-, ethyl-, propyl- and butylparaben which are medically accepted for parenteral administration.
 55 However, very often in accordance with the invention preservatives will not be required since the compositions may be sterilized by autoclaving without essentially reducing their stability. If desired, the pharmaceutical compositions of the present invention may also comprise an osmotic pressure regulator such as mannitol or glycerin, glycerin being preferred for parenteral administration and mannitol for oral

administration. The compositions of the present invention may also comprise an antioxidant such as α -tocopherol.

The compositions of the present invention are suitable for the administration of hydrophobic drugs, i.e. drugs having a low water solubility. Examples of such drugs are: Hydrophobic or lipophilic antibiotic drugs such as amphotericin B; hydrophobic and lipophilic narcotic drugs such as alkaloid bases, e.g. morphine-base base; hydrophobic benzodiazepines such as diazepam, fluphenazine deconate and lorazepam; non-steroidal anti-inflammatory lipophilic drugs such as piroxicam and indomethacin; lipophilic steroids such as progesterone and testosterone propionate; lipophilic azoles such as miconazole and clotrimazole; lipophilic polypeptides such as cyclosporine; lipophilic sterols such as deoxycortone and calciferol; lipophilic cephalosporines; dimercaptol.

Where the drug in such compositions is Amphotericin B, its concentration is preferably about 0.015 - 0.15%, about 0.075% being particularly preferred. Where the drug in said composition is morphine-base-base, its concentration is preferably about 0.2-2%. Where the drug in said composition is either diazepam or indomethacin, its concentration is preferably about 0.1-1%, about 0.4-0.5% being especially preferred. Where the drug in said composition is ciclosporine, its concentration is preferably about 2-5%. Where the drug in said composition is miconazole, its concentration is preferably about 1-3%.

Generally, the oily droplets in the oil-in-water emulsion for medical use should preferably be small, i.e. below about 1 μm , preferably below 0.5 μm and desirably below 0.2 μm , since the smaller the droplets, the more stable in storage is the emulsion. The droplet size is furthermore of particular importance if the emulsion is to be used for parenteral administration, and especially for intravenous injections, since large droplets will not readily pass through small blood capillaries. The compositions of the invention are particularly suitable for obtaining such small oily droplet.

The compositions of the present invention may be prepared by a number of ways. By one preparation mode, an aqueous solution and an oily solution are separately prepared, the aqueous solution comprising the non-ionic surfactant, the said ionic surfactant and the phospholipids and optionally also an osmotic pressure regulator and a preservative, and the oily solution comprising the said oily carrier, the hydrophobic drug and optionally also an antioxidant. By a slight modification of this mode, the aqueous solution is prepared from two *a priori* prepared solutions, a first, alcoholic, solution containing the said ionic surfactants and the phospholipid and a second solution containing in water the non-ionic surfactant and if desired also the other optional ingredients mentioned above. The said aqueous solution is then prepared by mixing the first and the second solution, then removing the alcohol, for example by evaporation, to yield the said aqueous solution. This modified mode is suitable for the preparation of composition of the invention in which the drug is for example diazepam or miconazole and the like.

The aqueous solution and the oily solution are then mixed with one another. However, the so-obtained mixture does not yet consist of sufficiently small droplets, the size of which (obtained after mixing with a magnetic stirrer) is about 10 μm . The droplet size of the inventive composition may then be decreased by the use of emulsification equipment such as Ultra Turrax (Jankl and Kunkel, Staufen, FRG), which yields droplets having an average diameter of about 1.1 μm , or of a high shear mixer, e.g. Polytron (Kinematica, Lucerne, Switzerland) which yields droplets having an average diameter of about 0.65 μm .

Especially small droplets are obtained in the inventive compositions when utilizing a two-stage pressure homogenizer in which the crude dispersion is forced under high pressure through the annular space between a spring loaded valve and the valve seat, the second stage being in tandem with the first so that the emulsion is subjected to two very rapid dispersion processes. An example of such an apparatus is the Gaulin Homogenizer (APV Gaulin, Hilversum, The Netherlands). Use of such an apparatus in accordance with the invention yields compositions in which the droplets have an average diameter of about 0.27 μm with a relatively small deviation.

Even smaller droplets may be obtained in accordance with the invention when the emulsification process combines the use of both a Polytron-type high shear mixer followed by homogenization. The droplet size which is obtained in such a combination is about 0.1-0.15 μm . Achieving of such a droplet size is highly important because of the profound effect this has on the increase in the composition's stability and such is a result of both the ingredients used for the preparations of the composition, i.e. MCT oil and optionally vegetable oil, phospholipid, non-ionic surfactant and said ionic surfactant as well as the method of preparation as described above.

The present invention provides also a novel mode (to be referred to hereinafter as the "inventive mode") for the preparation of the above compositions. The inventive mode is particularly suitable for the preparation of compositions in accordance with the invention in which the drug is both hydrophobic and has a poor oil solubility, i.e. drugs which are present in the compositions, predominantly in the intermediate phase. However, it should be noted that the inventive mode, as will be explained below, is principally

suitable also for the preparation of compositions of the invention in which the hydrophobic drug is lipophilic, namely oil soluble and thus present in the compositions predominantly in the oily phase.

In accordance with the inventive mode the compositions are prepared by mixing together a liposome mixture and an oily mixture, each one prepared separately beforehand. The liposome mixture comprises all the ingredients which in the final composition do not form part of the oily phase, namely the phospholipids, the non-ionic surfactant, the said ionic surfactant, and, if desired, also the optional osmotic pressure regulator and the preservative. Where the drug has a poor oil solubility, such as Amphotericin B, it is also included in the liposome mixture. The preparation of the liposome mixture from these ingredients may be carried out by means known per se.

The oily mixture comprises the said oily carrier and, if desired, also the optional anti-oxidant. Where the drug is lipophilic, it is also included in the oily mixture.

After the liposome mixture is mixed together with the oily mixture, an emulsion is formed having relatively large droplets, e.g. about 10 μm , which is further processed in a similar manner as described above in connection with the first preparation mode, until an emulsion having fine homogenous droplets is obtained.

Compositions of the present invention which contain heat resistant drugs remarkably withstand sterilization in an autoclave. Such sterilization is easier to perform and more practical on industrial scales, than sterilization by filtration as described in EP-A2-214561 and EP-A3-245313. However, where the drugs are heat sensitive, such as Amphotericin B, standard aseptic conditions should be employed.

In the following Examples, the preparation of some specific compositions according to the present invention is described, it being understood that these Examples are for illustrative purposes only and that the invention is not limited thereto.

25 DESCRIPTION OF THE DRAWINGS

In the following description, reference will at times be made to the annexed drawings in which:

Fig. 1 is a schematic representation of the process for extracting pure phospholipids from crude egg yolk phospholipids;

Fig. 2 is a schematic representation of an emulsion preparation process;

Fig. 3 shows droplet size distribution in a composition not containing any drug (a) and in a composition containing the drug Amphotericin B (b);

Fig. 4 shows the droplet size distribution after three months storage at 4° C of compositions containing the drug amphotericin B (a) and compositions containing no lipophilic drug (b).

Fig. 5 shows the survivability with time of *Candida albicans* infected mice after injection of Fungizone (trade name), an amphotericin B emulsion in accordance with the invention and of saline.

40 EXPERIMENTAL METHODS

Measurement of emulsion properties in tests reported in some of the following Examples was performed as follows:

45 A. Analysis of Particle Size

The droplet size distribution of the Amphotericin B emulsions was determined by two complementary methods, namely by the photon correlation spectroscopy (PCS) using a computerized laser light scattering apparatus, considered the most appropriate for studying droplet size below 1 μm (Malvern system, Malvern, England), and the computerized laser inspection system which can measure droplet sizes above 0.6 μm (Galai Cis-1, Migdal Haemek, Israel). In the Malvern system, each emulsion sample was diluted to the appropriate concentration with a filtered isotonic solution (2.25% glycerin in water) before measurement at 25° C. Duplicate samples were taken and each sample was analysed 10 times. In the Galai system, the samples were also diluted with 2.25% glycerin and two counts were made on each sample. The dilution was needed to increase viscosity thereby reducing the velocity movement of the droplets towards the surface.

The advantage of the Galai Cis-1 system over the widely used Coulter Counter system is demonstrated by the fact that there is no need for an electrolyte solution that can affect the stability of the emulsions.

Both methods were needed since none of them was able to measure accurately droplet size of the entire range of 100 to 3000nm. At most cases, the droplet size of the tested emulsions ranged from 100 to 400nm. However, when emulsions were subjected to accelerated stability tests, higher mean droplet size could have been in theory expected, and therefore it was necessary to measure diameters in the range of 0.6 to 2.0 μm . Thus, the droplet size distribution of emulsions which underwent stress conditions was analyzed using both the Galai Cis-1 and the Malvern systems.

B. Measurements of Zeta Potential

The zeta potential was measured using the moving boundary electrophoresis technique, which has been shown to yield accurate electrophoretic mobility data. Measurement of electrophoretic mobility and conversion of electrophoretic mobility data to zeta potential, were performed similarly as previously described (Benita, et. al., 1984, J.Pharm. Sci, 73: 1751-1755.), the electrolyte consisted of an aqueous solution containing 1% glycerol and 0.75% Pluronic F-68 which helped to stabilize the moving boundary (preventing the free diffusion of the droplets due to osmotic pressure which occurs in normal aqueous media) without altering the electrophoretic mobility.

Each emulsion sample was diluted with 9 parts of water to 1 part of sample, prior to examination. In order to confirm the measurements reliability various commercial Intralipid (trade name manufactured by Kabi-Vitrum) fat emulsions (which are emulsion used for nutrition, and comprise 10-20% soy oil, phospholipids and glycerin) were measured by this technique and were found to be identical, within the limits of experimental error, to corresponding zeta potential values published in the literature. Further confirmation of the reliability of this method came from the repeatability of results.

C. pH Measurement

The pH of the emulsion samples was measured using a pH meter (radiometer pH M63, Copenhagen, Denmark).

Example 1: Preparation of an emulsion containing diazepam

A) Oily, alcoholic and aqueous solutions were separately prepared as follows (all amounts below are given in % w/v in relation to the final total emulsion volume):

I. Oily Solution:

The oily solution consisted of the following ingredients:

1) 10% MCT (Societe Industrielle de Oleagineux, St. Laurent, Blangy, Francy), and 10% purified soybean oil (Bertin, Courbevoie, France), which was refrigerated for about a week to remove waxy substances.

2) 0.05% α -tocopherol (Sigma Chemicals, St. Louis, MO, U.S.A.).

3) 0.5% diazepam (Teva Pharmaceuticals Inc., Kfar Saba, Israel).

II. Alcoholic Solution

The alcoholic solution consisted of the following ingredients:

1) 0.5% Deoxy cholic acid - D.C.A. (Sigma, St. Louis, MO, U.S.A.).

2) 1.0% purified fractionated egg yolk phospholipids, prepared from crude egg yolk phospholipids (Sigma Chemicals, St. Louis, MO, U.S.A.), in accordance with a modification of an earlier technique reported by Schuberth and Wrethind (Acta. Chir. Scand. Suppl. 278: 1-21, 1961), as represented schematically in Fig. 1 of the annexed drawings. The purification generally consisted of four extraction cycles with petroleum ether 80-100 as extraction solvent. These purified phospholipids consisted mainly of phosphatidylcholine and phosphatidylethanolamine, together with small quantities of phosphatidylserine, phosphatidylinositol and phosphatidic acids (verified by standard TLC procedure).

III. Aqueous Solution:

The aqueous solution consisted of the following ingredients:

- 1) 2% Poloxamer (Pluronic® F-68, BASF, Ludwigshafen, FRG)
- 2) 2.25% glycerin (Merck, Darmstadt, FRG).
- 3) 0.2% methyl paraben (methyl p-hydroxybenzoic ester; Sigma Chemicals, St. Louis, MO., U.S.A.) and 0.075% of butyl paraben (butyl p-hydroxybenzoic ester; Sigma).
- 4) Double distilled water (DDW) added up to 100%.

Ingredient (3) was not always present and was only added when no autoclaving was to be applied.

The alcohol solution was admixed with the aqueous solutions and the alcohol was removed by evaporation. The so obtained solution is the one which is hereinafter referred to as the "aqueous solution".

Emulsion Preparation:

The whole process for the preparation of the emulsion was conducted under nitrogen atmosphere and under aseptic conditions. This process is schematically represented in Fig. 2 of the annexed drawings.

Both solutions were appropriately filtered using 0.22 μm millipore membrane filter. After filtration, the aqueous and the oily solutions were heated separately to 70 °C and dispersed by a magnetic stirrer. Further heating was applied while mixing until the temperature reached 85 °C. At this temperature, the two solutions were mixed and emulsified during 5 min. using a high shear mixer. [Polytron (trade name) Kinematica, Lucerne, Switzerland]. The resulting coarse emulsion was cooled rapidly below 20 °C. A fine monodispersed emulsion (i.e. having a small range of droplet sizes) was then achieved following 20 homogenization cycles, using a two-stage homogenizing valve assembly (Gaulin homogenizer, APV Gaulin, Hilversum, The Netherlands), at a temperature of 40-70 °C under pressure of about 8500 psi.

Following the homogenization, the pH was adjusted to 8.0 using a 10% sodium hydroxide solution and the emulsion was filtered using a 1 μm filter, to discard the coarse droplets which were generated during the emulsification and homogenization processes and also other debris.

Samples of the so filtered fine clean emulsion were stored in 10 ml brown ampoules and no phase separation was observed over periods of more than fourteen months.

Example 2: Preparation of an emulsion containing diazepam

In a similar manner to that described in Example 1, diazepam containing emulsions were prepared, replacing, however, the purified fractionated egg yolk phospholipids (ingredient 3 of the oily solution) with Ovothin 160 (Lucas Meyer, Hamburg, Federal Republic of Germany).

Similarly as in Example 1, no phase separation was seen even after storage of more than twelve months.

Example 3: Stability of the emulsion

The stability of the emulsions of Examples 1 and 2 was determined by measuring various parameters of the emulsion on various time intervals following their preparation. The emulsions were stored at 4 °C. Essentially no change in either pH, zeta potential, mean droplet size, droplet size distribution and drug content was observed for more than fourteen months after preparing the emulsions. The stability tests are still under evaluation.

Some batches of the above emulsions were subjected, following their preparation, to a thermal shock by subjecting them to autoclaving - 1.1atm., 121 °C, 15 min. Such a thermal shock is considered in the art as an effective accelerated test for assessing emulsion stability. However, surprisingly, even for such emulsions, no loss in stability was observed determined by measuring the same above parameters and similarly as above these emulsions retained their initial properties for more than four months after sterilization was performed.

Example 4 : Stability of emulsions prepared with either deoxycholic acid or sodium deoxycholate

The emulsion of Example 2 was successively modified to give four different formulations as shown in the following Table I:

TABLE I

Ingredients	Amount (%)	Formulations			
		1	2	3	4
Soybean Oil		-	10.0	-	10.0
MCT oil		20.0	10.0	20.0	10.0
α -tocopherol	0.02	+	+	+	+
Ovothin 160	1.0	+	+	+	+
Diazepam	0.5	+	+	+	+
Pluronic F-68	2.0	+	+	+	+
SDC.*		-	-	1.0	1.0
DCA.**		0.5	0.5	-	-
Glycerol	.25	+	+	+	+
Water to	100	+	+	+	+

* SDC. - Sodium deoxycholate

** DCA. - Deoxy cholic acid

The zeta potential and the droplet size of the various formulations is shown in the following Table II:

TABLE II

Formulation	Droplet size (nm)		Zeta Potential (mv)
	mean	S.D.*	
1	112	27.9	48
2	117	29.8	45
3	125	31.2	35
4	137	36.8	32

* S.D. - standard deviation

It can be seen that the compositions comprising MCT oil as the sole oily carrier had improved properties as compared to those prepared with equal concentrations of both MCT and vegetable oil. Additionally, the results show that emulsions comprising DCA had smaller, more uniform droplets and higher zeta potential than compositions comprising SDC. Hence the preference in utilizing DCA over SDC.

Example 5 : Synergism between phospholipids, non-ionic surfactant and deoxycholic acid in diazepam containing emulsions

The emulsion of Example 2 (without α -tocopherol) was successively modified to give emulsions of different formulations as shown in Table III below:

TABLE III

Ingredient	Concentration in % (w/v)	FI	FII	FIII	FIV	FV	FVI	FVII
Soybean Oil	10.0	+	+	+	+	+	+	+
MCT Oil	10.0	+	+	+	+	+	+	+
Diazepam	0.5	+	+	+	+	+	+	+
Ovothin 160	1.0	+	+	+	-	+	-	-
Poloxamer F-68	2.0	+	+	-	+	-	+	-
Deoxycholic acid	0.5	+	-	+	+	-	-	+
Glycerin	2.25	+	+	+	+	+	+	+
Distilled water to 100.0		+	+	+	+	+	+	+

All formulations were prepared on the same day with the same reagents and using the same preparative procedure, as described in Example 1. Following preparation the emulsions were sterilized by autoclaving (121 °C, 1.1 atm, 15 min.).

The emulsions were first checked visually to determine the degree of creaming and those compositions that remained stable after 1 week were checked further to determine the droplet size and zeta potential.

The following results were obtained:

Formulation FI:

This emulsion, which was the one of Example 1, was the most stable one and it remained stable for over fourteen months, and no creaming and phase separation were observed.

Zeta potential of this emulsion was measured to be -45mv and the mean droplet diameter was measured to be about 117 nm.

Formulation FII:

The emulsion was unstable and 3-4 weeks after its preparation phase separation was observed. The phase separation was evident by the appearance of oil drops on the upper surface of the emulsion.

Formulation FIII:

Phase separation was observed within one day after preparation of the emulsion.

Formulation FIV:

Creaming was observed within a day of preparation of the emulsion. There was a smaller proportion of drops having a larger diameter, which caused creaming, which developed in the course of time to a total phase separation.

Formulation FV:

The emulsion was unstable and total phase separation was observed within 2 days.

Formulation FVI:

Creaming was observed in the emulsion within a day of its preparation. The degree of creaming increased in the following days resulting finally in a complete phase separation.

Formulation VII

The emulsion was very unstable and phase separation was already observed in the course of its preparation.

SUMMARY:

The emulsion of Formulation FI is the only one containing all the ingredients. The many-fold increase in stability over any of the emulsions of Formulations FII to FVII is attributed to the synergistic action which the oils, phospholipids, non ionic surfacants and deoxycholic acid have on long term stability of the composition.

Example 6 : The effect of a thermal shock on a diazepam containing emulsions

The emulsions of Example 4 were subjected to a thermal shock by sterilizing in an autoclave (15 min, 121°, 1.180 atm). The mean droplet size (M.D.S.) and the standard deviation (S.D.) were determined before and after autoclaving and the results are given in the following Table IV:

TABLE IV

Formulation No.	Before autoclaving		After autoclaving	
	M.D.S.	S.D.	M.D.S.	S.D.
1	112	27.9	112	29.6
2	117	29.8	126	31.2
3	125	31.2	150	39.4
4	137	36.8	135	37.6

The above results show clearly that sterilization had very little effect on droplet size of the emulsions and this remarkable result is in contrast to the instability of hitherto known emulsions of the oil-in water type.

Example 7 : Stability to autoclaving of a micronazole base containing emulsions

In a similar manner to that described in Example 1 an emulsion comprising the following ingredients was prepared (% w/v): Miconazole base - 1.0; MCT oil - 20.0; Ovothin 160 -1.0; α -Tocopherol - 0.02; Pluronic F-68 - 2.0; DCA -0.5; Glycerol 2.25; and Water to 100.

The droplet size and standard deviation was measured before and after sterilization in an autoclave as in Example 6 and the results are shown in the following Table V:

TABLE V

Before autoclaving		After autoclaving	
M.D.S.	S.D.	M.D.S.	S.D.
135	45.9	141	30.0

Here again, the surprising stability of the emulsion to autoclaving is demonstrated.

Example 8: Preparation of an emulsion containing amphotericin B

Amphotericin B was dissolved in methanol (0.8 mg/ml) by bath sonication (15 min). Phospholipids E-80 (containing mainly 80% phosphatidylcholine and 8% phosphatidyl ethanolamine according to manufacturer specifications) were dissolved in chloroform. Both solutions were mixed and filtered through a combined filtering system comprising a fiber glass prefilter (GF.92, Schleicher and Schuel, FRG) and 0.45µm regenerated cellulose membrane filter (RC 5, Schleicher and Schuel, FRG), for removing pyrogens and aggregates. The resulting clear lipid solution was deposited as a thin film on the walls of a round-bottom flask by rotary evaporation under reduced pressure at 40° C. The aqueous phase comprising the poloxamer, sodium deoxycholate and glycerin was filtered through a 0.22 µm Millipore (trade name, manufactured by Millipore, Bedford, Mass., U.S.A.) filter, poured into the flask and the dispersion was sonicated until a homogeneous liposomal mixture was achieved.

MCT oil, filtered through 0.22 µm Millipore filter and containing α-tocopherol was heated to 70° C and then admixed into the liposomal mixture heated to 45° C and dispersed therein by a magnetic stirrer. Emulsification was carried out while maintaining the same temperature using a high shear mixer, Polytron (Kinematica, Luzern, Switzerland). The resulting coarse emulsion was cooled rapidly. A fine monodispersed emulsion was achieved using a two stage homogenizer (APV Gaulin, Hilversum, The Netherlands).

Finally, the pH of the emulsion was adjusted to 8.0 with unbuffered 10% sodium hydroxide solution and the final emulsion was filtered through a 0.45 µm Millipore filter to discard coarse droplets and debris generated during the emulsification and homogenization processes.

All the processing operations were carried out under aseptic conditions. The sterility of the emulsions was assessed using the Bactec 46 apparatus (Johnson Laboratories, Towson, MD). This instrument is used to test inoculated Bactec culture vials for the presence of radioactive Carbon dioxide (¹⁴CO₂) in the vials. Should a high level of ¹⁴CO₂ be present in vials used for culturing aerobic or anaerobic organisms, it indicates that there were viable microorganisms in the original inoculum. The negative results obtained using this technique showed that the emulsions were sterile.

The relative amounts of the various ingredients in the final emulsions were as follows:

0.075% amphotericin B, 20% MCT oil, 0.5% phospholipid E 80, 2.0% poloxamer, 1.0% sodium deoxycholate, 2.25% glycerine, 0.02% α-Tocopherol and bi-distilled water 200%.

For the purpose of various comparative tests to be reported below, also a plain emulsion, namely one which did not contain any lipophilic drug was prepared under identical experimental conditions but with the absence of amphotericin.

Example 9: Evaluation of the properties of an amphotericin B containing emulsion

The emulsion of Example 8 was evaluated and found to have a mean droplet size of about 100 nm as shown in Fig. 3. Furthermore, as may be seen in this Figure, no notable difference was found in the droplet size between the amphotericin and the plain emulsion.

This is further shown in the following Table VI:

TABLE VI

EMULSIONS	DROPLET SIZE			ZETA POTENTIAL (mv)	pH
	MEAN	S.D.(*)	POLY D.(**)		
PLAIN	103	29.8	0.122	-35.3	8.0
WITH AMPHOTERICIN	97.5	23.8	0.130	-32.4	8.0

(*) S.D. - Standard deviation

(**) Poly D - polydispersity, a factor reflecting the homogeneity of the population

As may be seen from Table VI, the droplet sizes of the two emulsions were very homogeneous. Furthermore, there was also no notable difference in the zeta potential of the two emulsions.

The amphotericin emulsion of Example 8 was stored for a period of three months at 4° C. After such a storage period, the zeta potential, the pH as well as the particle size distribution of both types of emulsion remained practically unchanged. Furthermore, the amount of Amphotericin, determined by HPLC as known per se, did not change for up to three months storage at 4° C, indicating that the stability of amphotericin was not affected by its incorporation in the emulsion.

The amphotericin emulsion was subjected to an accelerated shaking stability test, at a shaking rate of 100 strokes per minute at 25° C for forty eight hours. No droplets having diameter above 1µm were detected by the Galai Cis-1 system while a slight and insignificant increase in the droplet size of the emulsion was noted with the Malvern PCS method. These results indicate that the amphotericin emulsion remained stable in spite of the mechanical stress.

The droplet size distribution of amphotericin emulsion and plain emulsion after three months' storage at 4° C is shown in Fig. 4. It may be seen that there is no difference between the size distribution of the two emulsions and by comparing these results with those of Fig. 3, it may be seen that there is no substantial difference in the droplet size distribution after storage.

Example 10: Animal studies with amphotericin formulations

Thirty balb/c mice (weighing about 20g) were injected through the tail vein with 5×10^5 of *Candida albicans*, strain 562, in 0.1 ml of saline. This dose was shown to cause a total death of the infected mice within five to ten days.

Forty eight hours after the infection was initiated each mouse received a post infection treatment. The mice were divided into three groups of ten mice, each group receiving a different treatment. The treatment consisted of an injection of 0.1 ml into the tail vein of one of the following formulations:

a) Fungizone which is a commercial amphotericin B formulation, (trade name manufactured by E.R. Squib & Sons Ltd) at an amount of 0.4 mg/kg of amphotericin; proper dilution in order to achieve the aforementioned amount was achieved in accordance with manufacturers' specification;

b) An emulsion of Example 8, at an amount of 0.4 mg/kg; dilution of the batch formulation in order to achieve the aforementioned amount was performed with a "plain emulsion" (see Example 8); c) Saline.

The number of surviving animals in each group was recorded daily, and the survival results are shown in Fig. 5.

It can be seen that all the untreated infected control animals died seven days after *Candida albicans* inoculation. All the infected mice which were treated with Fungizone died from infection 11-19 days after the injection while a much prolonged survival rate was observed in the animals injected with the amphotericin emulsions in accordance with the invention, and 50 days after inoculation, 55% of the mice were alive and appeared in a good condition.

These results demonstrate the improved medical properties of emulsions in accordance with the present invention.

Claims

1. A pharmaceutical composition being an oil-in-water type emulsion comprising an effective amount of a lipophilic drug, characterised in that it comprises about 3-50% of an oily carrier consisting of mid chain triglyceride (MCT) oil, optionally in combination with vegetable oil, about 0.05-20% of a phospholipid, about 0.03-10% of a non-ionic surfactant, and about 0.05-5% of an ionic surfactant selected from the group consisting of bile-duct surface active agent, cholic acid and their derivatives.

2. A composition according to Claim 1 comprising MCT oil as the sole oily carrier.

3. A composition according to Claims 1 or 2 in a form suitable for parenteral administration.

4. A composition according to Claims 1 or 2 in a form suitable for topical administration.

5. A composition according to Claims 1 or 2 in a form suitable for oral administration.

6. A composition according to Claims 1 or 2 in a form suitable for ocular administration.

7. A composition according to Claim 3, in which the relative proportion of the non-aqueous phase which comprises said oily carrier, phospholipids, non-ionic surfactant and said ionic surfactant does not exceed 30%.

8. A composition according to Claim 1 in which the relative proportion of said non-aqueous phase does not exceed 25%.

9. A composition according to any one of the preceding claims, wherein the relative proportion of the

oily carrier is about 10-20%.

10. A composition according to any one of the preceding claims, wherein the relative proportion of the phospholipid is about 0.5-2%.

11. A composition according to Claim 10, wherein the relative proportion of the phospholipid is about 0.75%.

12. A composition according to any one of the preceding claims, wherein the relative proportion of the non-ionic surfactant is about 0.5-3%.

13. A composition according to any one of the preceding claims, wherein the relative proportion of the said non-ionic surfactant is about 3%-10%.

14. A composition according to Claim 13, wherein the relative proportion of the said ionic surfactant is about 4-6.6%.

15. A composition according to any one of preceding claims, wherein the MCT oil is selected from TCM (trade name) and Miglyal 812 (trade name).

16. A composition according to any one of Claims 1 and 3-14, wherein the vegetable oil is selected from the group consisting of soybean oil, cotton seed oil, olive oil and sesame oil.

17. A composition according to any one of the preceding claims, wherein the phospholipids are selected from the group consisting of Epikuron (trade name), Ovothin 160 (trade name), purified phospholipids and Phospholipids F-80 (trade name).

18. A composition according to any one of the preceding claims, wherein the non-ionic surfactant is Poloxamer (trade name).

19. A composition according to any one of the preceding claims, wherein the said ionic surfactant is selected from the group consisting of cholic acid, deoxycholic acid and their surface active derivatives and salts.

20. A composition according to Claim 19, wherein the said ionic surfactant is selected from the group consisting of cholic acid, deoxycholic acid and their sodium salt.

21. A composition according to any one of the preceding claims, wherein said lipophilic drugs are selected from the group consisting of hydrophobic or lipophilic antibiotics or narcotic drugs, hydrophobic benzodiazepines, non-steroidal anti-inflammatory lipophilic drugs, lipophilic steroids, lipophilic azoles, lipophilic polypeptides, lipophilic steroids, lipophilic cephalosporines and dimercaptol.

22. A composition according to Claim 21, wherein said drug is selected from the group consisting of amphotericin B, morphine-base, diazepam, fluphenazine deconate, lorazepam, piroxicam, indomethacin, progesterone, testosterone propionate, miconazole, clotrimazole, cyclosporine, deoxycortone, calciferol, cephalosporine and dimercaptol.

23. A composition according to Claim 22, wherein said lipophilic drug is amphotericin B.

24. A composition according to Claim 22, wherein said lipophilic drug is diazepam.

25. A composition according to Claim 22, wherein said lipophilic drug is morphine, indomethacin, cyclosporine or miconazole.

26. A composition according to any one of the preceding claims, wherein the mean oily droplet diameter is below 0.2 μ m.

27. A composition according to Claim 26, wherein the mean oily droplet diameter is about 0.1-0.15 μ m.

28. A process for the preparation of a composition of the oil-in-water type emulsion comprising an effective amount of a lipophilic drug, about 3-50% of an oily carrier consisting of MCT oil, optionally in combination with vegetable oil, about 0.05%-20% of phospholipid, about 0.03-10% of a non-ionic surfactant, and about 0.05-5% of an ionic surfactant selected from the group consisting of bile-duct surface active agent, cholic acid and their derivatives, which process comprises:

(a) preparing a liposome mixture comprising the phospholipids, the non-ionic surfactant, the said ionic surfactant, and where the drug has a poor oil solubility, also comprising said drug;

(b) preparing an oily mixture comprising said oily carrier, and where the drug is lipophilic, also comprising said drug;

(c) mixing said liposome mixture with said oily mixture, whereby said emulsion is obtained.

29. A process according to Claim 28, wherein the so obtained emulsion is subjected to further stages in which smaller droplets in the emulsion are obtained and/or the homogeneity of the droplets' size is increased.

30. A process according to Claim 28 or 29, wherein said drug has a poor oil solubility.

31. A process according to Claim 30, wherein said drug is amphotericin B.

Claims for the following Contracting States: ES, GR

1. A process for the preparation of a composition of the oil-in water type emulsion comprising an effective amount of a lipophilic drug, about 3 to 50% of an oily carrier consisting of mid chain triglyceride (MCT) oil, optionally in combination with vegetable oil, about 0.05 to 20% of phospholipid, about 0.03 to 1.0% of a non-ionic surfactant, and about 0.05 to 5% of an ionic surfactant selected from the group consisting of bile-duct surface active agents, cholic acid and their derivatives, which process comprises:
 - (a) preparing a liposome mixture comprising the phospholipids, the non-ionic surfactant, the said ionic surfactant, and where the drug has a poor oil solubility, also comprising said drug;
 - (b) preparing an oily mixture comprising said oily carrier, and where the drug is lipophilic, also comprising said drug;
 - (c) mixing said liposome mixture with said oily mixture, whereby said emulsion is obtained.
2. A process according to Claim 1, wherein the so obtained emulsion is subjected to further stages, in which smaller droplets in the emulsion are obtained and/or the homogeneity of the droplets' size is increased.
3. A process according to Claim 1 or 2, wherein said drug has a poor oil solubility.
4. A process according to Claims 1 to 3, comprising MCT oil as the sole oily carrier.
5. A process according to Claims 1 to 3 or 4 in a form suitable for parenteral administration.
6. A process according to Claims 1 to 4 in a form suitable for topical administration.
7. A process according to Claims 1 to 4 in a form suitable for oral administration.
8. A process according to Claims 1 to 4 in a form suitable for ocular administration.
9. A process according to Claim 5, in which the relative proportion of the non-aqueous phase which comprises said oily carrier, phospholipids, non-ionic surfactant and said ionic surfactant does not exceed 30%.
10. A process according to Claim 9, in which the relative proportion of said non-aqueous phase does not exceed 25%.
11. A process according to any one of the preceding claims, wherein the relative proportion of the oily carrier is about 10 to 20%.
12. A process according to any one of the preceding claims, wherein the relative proportion of the phospholipid is about 0.5 to 2%.
13. A process according to Claim 12, wherein the relative proportion of the phospholipid is about 0.75%.
14. A process according to any one of the preceding claims, wherein the relative proportion of the non-ionic surfactant is about 0.5 to 3%.
15. A process according to any one of the preceding claims, wherein the relative proportion of the said non-ionic surfactant is about 3 to 10%.
16. A process according to Claim 15, wherein the relative proportion of the said ionic surfactant is about 4 to 6.6%.
17. A process according to any one of the preceding claims, wherein the MCT oil is selected from TCM (trade name) and Miglyal 812 (trade name).
18. A process according to any one of Claims 1 and 5 to 16, wherein the vegetable oil is selected from the group consisting of soybean oil, cotton seed oil, olive oil and sesame oil.
19. A process according to any one of the preceding claims, wherein the phospholipids are selected from the group consisting of Epikuron (trade name), Ovothin 160 (trade name), purified phospholipids and Phospholipids F-80 (trade name).
20. A process according to any one of the preceding claims, wherein the non-ionic surfactant is Poloxamer (trade name).
21. A process according to any one of the preceding claims, wherein the said ionic surfactant is selected from the group consisting of cholic acid, deoxycholic acid and their surface active derivatives and salts.
22. A process according to Claim 21, wherein the said ionic surfactant is selected from the group consisting of cholic acid, deoxycholic acid and their sodium salt.
23. A process according to any one of the preceding claims, wherein said lipophilic drugs are selected from the group consisting of hydrophobic or lipophilic antibiotics or narcotic drugs, hydrophobic benzodiazepines, non-steroidal anti-inflammatory lipophilic drugs, lipophilic steroids, lipophilic azoles, lipophilic polypeptides, lipophilic steroids, lipophilic cephalosporines and dimercaptol.
24. A process according to Claim 23, wherein said drug is selected from the group consisting of amphotericin B, morphine-base, diazepam, fluphenazine deconate, lorazepam, piroxicam, indomethacin, progesterone, testosterone propionate, miconazole, clotrimazole, cyclosporine, deoxycortone, calciferol, cephalosporine and dimercaptol.

25. A process according to Claim 24, wherein said lipophilic drug is amphotericin B.

26. A process according to Claim 24, wherein said lipophilic drug is diazepam.

27. A process according to Claim 24, wherein said lipophilic drug is morphine, indomethacin, cyclosporine or miconazole.

5 28. A process according to any one of the preceding claims, wherein the mean oily droplet diameter is below $0.2\text{ }\mu\text{m}$.

29. A process according to Claim 28, wherein the mean oily droplet diameter is about 0.1 to $0.15\text{ }\mu\text{m}$.

10

15

20

25

30

35

40

45

50

55

SCHEMATIC EGG YOLK PHOSPHOLIPID EXTRACTION PROCESS

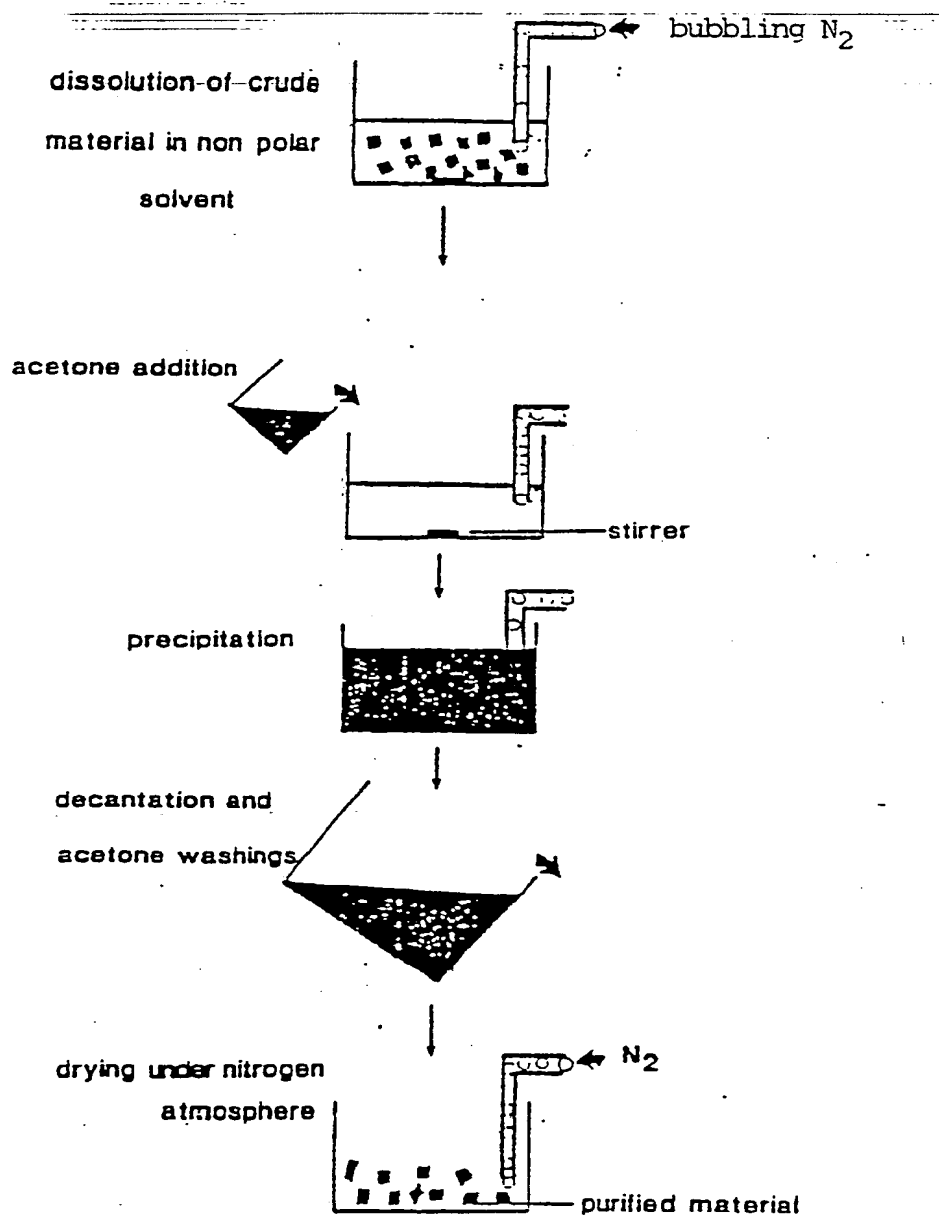


Fig. 1

SCHEMATIC EMULSION PREPARATION PROCESS

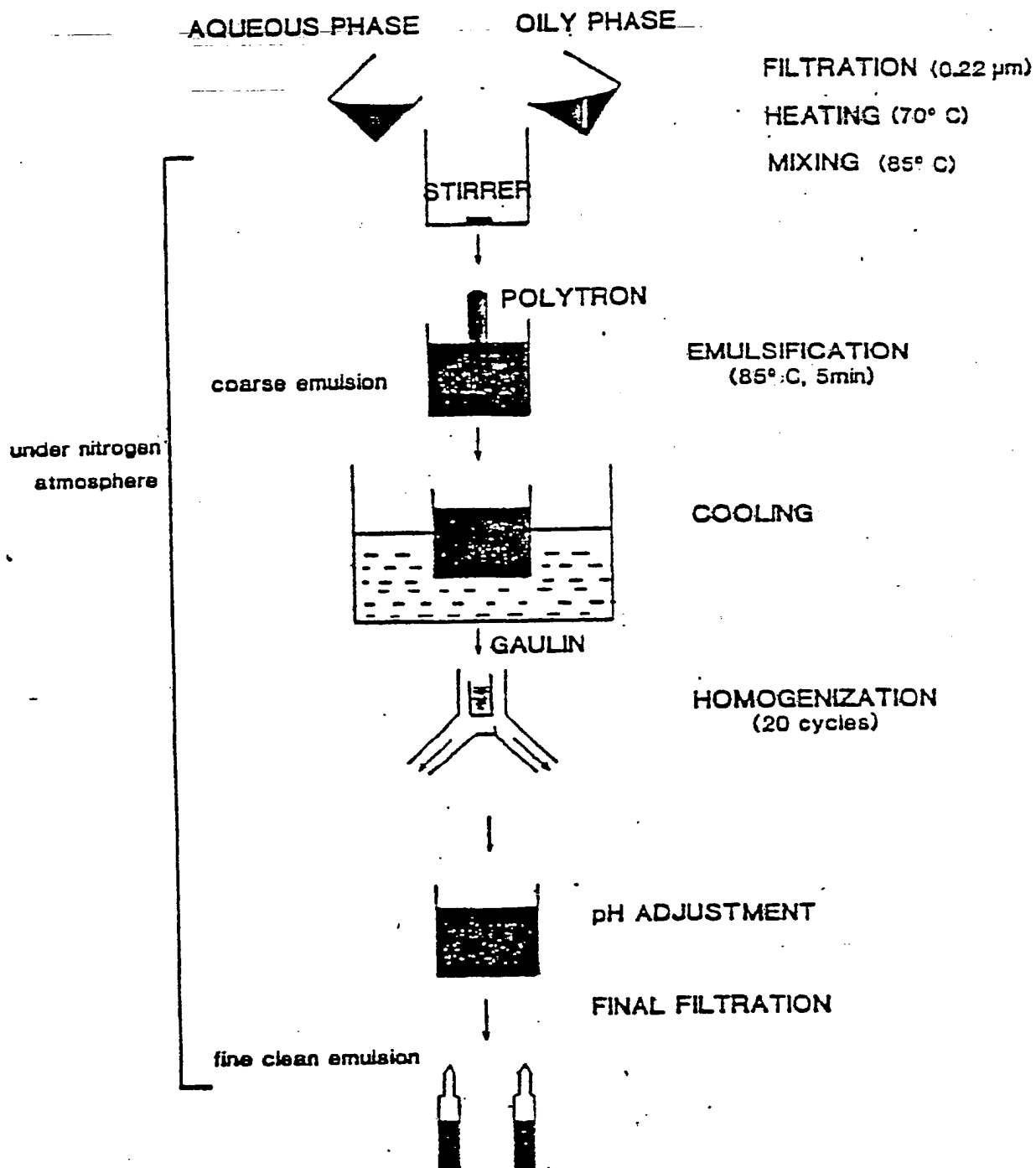
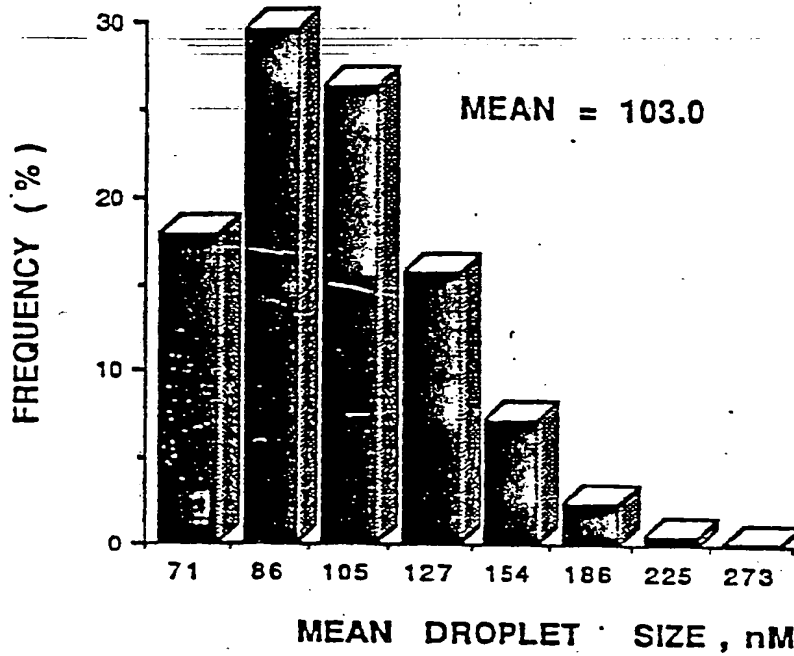


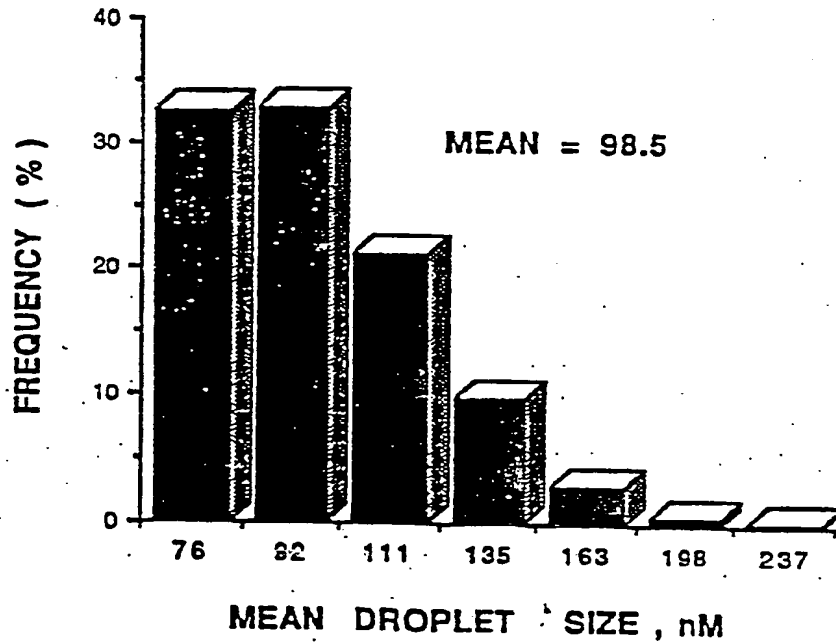
Fig. 2

DROPLETS SIZE DISTRIBUTION OF PLAIN EMULSION



A

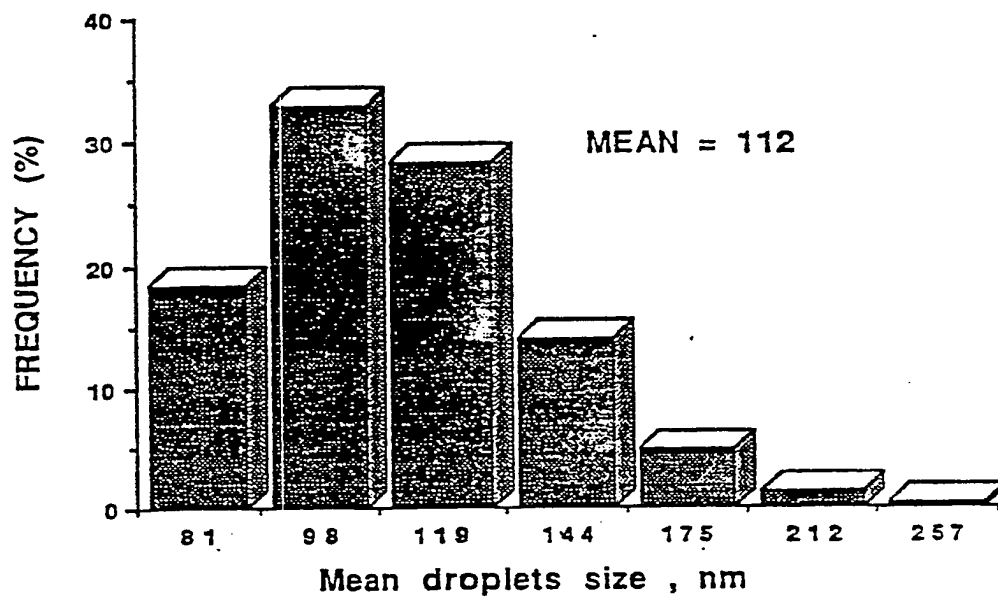
DROPLETS SIZE DISTRIBUTION OF AMPHOTERICIN EMULSION



B

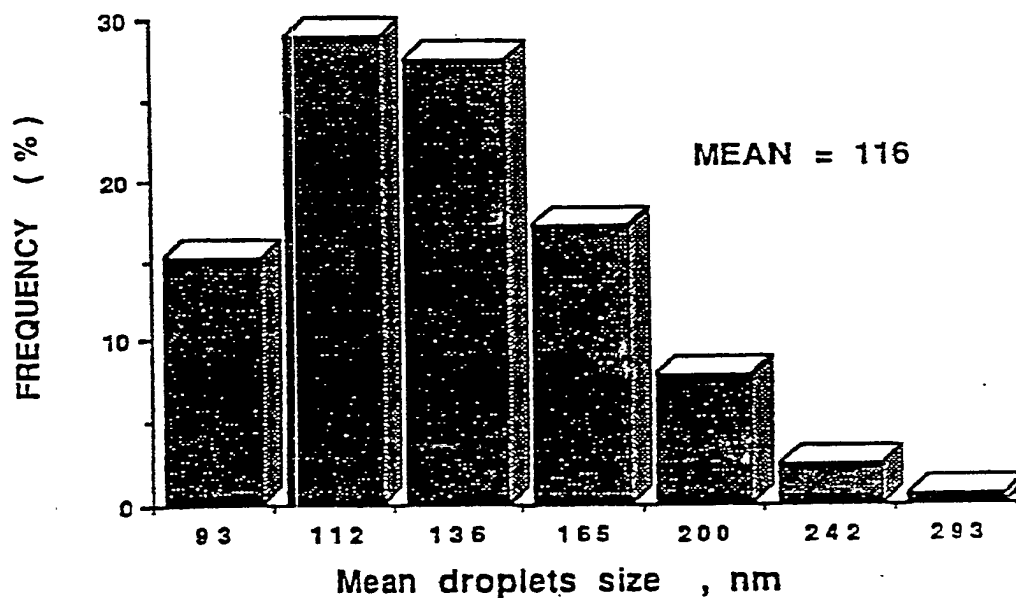
FIG.3

**DROPLETS SIZE DISTRIBUTION OF AMPOTERICIN EMULSION
AFTER 3 MONTHS STORAGE AT 4 C**



A

**DROPLETS SIZE DISTRIBUTION OF PLAIN EMULSION
AFTER 3 MONTHS STORAGE AT 4 C**



B

FIG.4

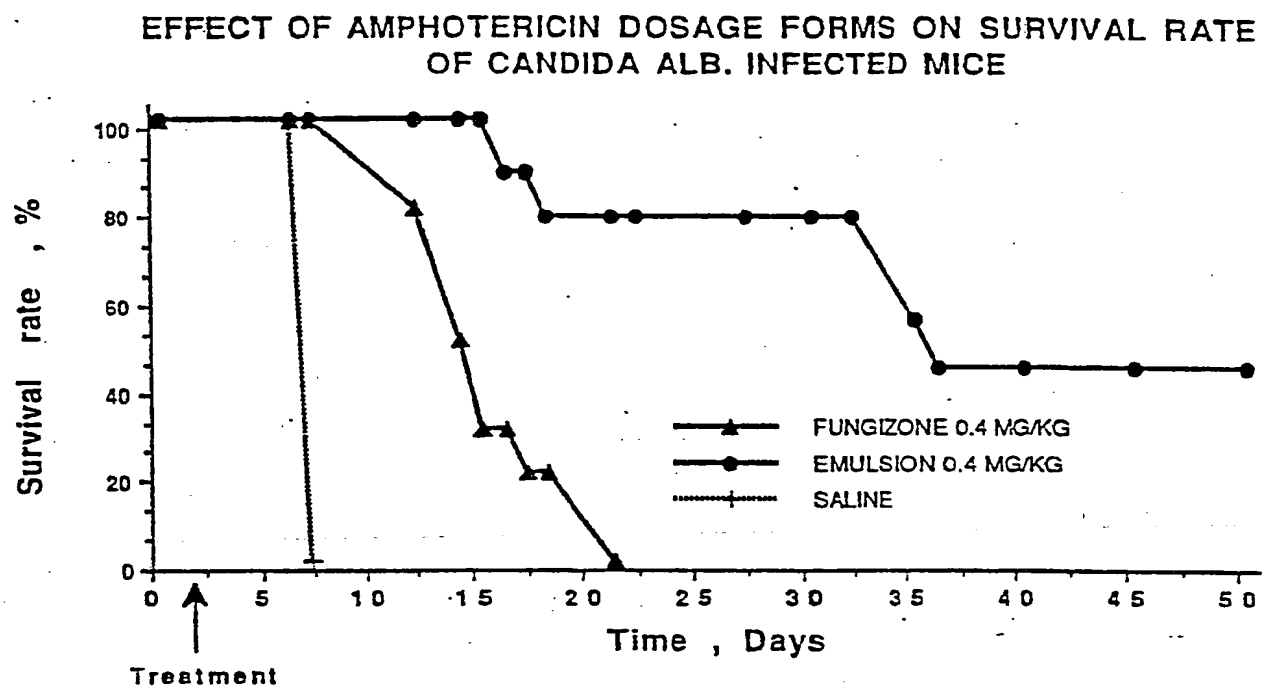


FIG. 5

THIS PAGE BLANK (USPTO)



①② **EUROPEAN PATENT APPLICATION**

②① Application number: 90106397.4

⑤① Int. Cl.5: **A61K 9/107, A61K 9/127**

②② Date of filing: **04.04.90**

③③ Priority: **05.04.89 IL 89856**
27.02.90 IL 93558

JERUSALEM
46 Jabotinsky Street
Jerusalem, 92 182(IL)

④③ Date of publication of application:
10.10.90 Bulletin 90/41

⑦② Inventor: **Simon, Benita**
33/3 Haarazim Strasse, Mevasseret Zion
Jerusalem 90805(IL)
Inventor: **Menashe, Levy**
15/5 Haarazim Strasse, Beith Hakerem
Jerusalem 96181(IL)

⑧④ Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

⑧⑧ Date of deferred publication of the search report:
24.04.91 Bulletin 91/17

⑦① Applicant: **YISSUM RESEARCH DEVELOPMENT**
COMPANY OF THE HEBREW UNIVERSITY OF

⑦④ Representative: **Vossius & Partner**
Siebertstrasse 4 P.O. Box 86 07 67
W-8000 München 86(DE)

⑤④ **Medicinal emulsions.**

⑤⑦ A pharmaceutical composition being an oil in water type emulsion which comprises an effective amount of a lipophilic drug is provided. The composition comprises a synergistic combination consisting of about 3-50% of an oily carrier consisting of MCT oil optionally in combination with vegetable oil, about 0.05%-20% of a phospholipid, about 0.03-10%

of a non-ionic surfactant, and about 0.05-5% of an ionic surfactant selected from the group consisting of bile-duct surface active agent, cholic acid and their derivatives.

Also provided is a novel method for preparing said composition.

EP 0 391 369 A3

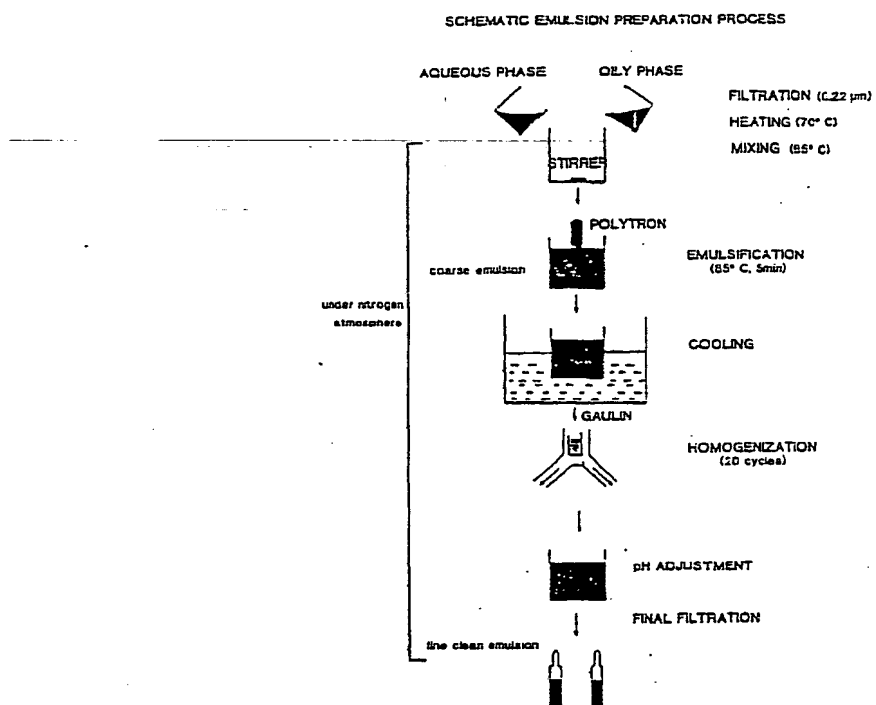


Fig. 2



European
Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 10 6397

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	US-A-4 115 313 (IRRING LYON et al.) * Claims 1,5,8; column 1, lines 12-14; column 2, lines 11-13,34-42,55-61; column 3, lines 27-42; column 4, lines 12-18; column 15, lines 59-68; column 16, lines 1-6; column 17, lines 55-68; column 18, lines 1-33 *	1,4,9-14, 16,19-21	A 61 K 9/107 A 61 K 47/28
E	EP-A-0 361 928 (SHISEIDO CO., LTD) * Claims 1,4; page 2, lines 6-7,38-47; page 3, lines 11-15,24-30,37-47; page 4, lines 18-22 *	1-3,9, 16-19, 21-23, 25-27	
X	PATENT ABSTRACTS OF JAPAN, vol. 5, no. 200, 18th December 1981, page 102 C 84; & JP-A-56 122 309 (TEIJIN K.K.) 25-09-1981 * Abstract *	1,16	
A	WO-A-8 901 327 (PARFUMS CHRISTIAN DIOR) * Example 15 *	28	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			A 61 K
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		31 January 91	VENTURA AMAT A.
CATEGORY OF CITED DOCUMENTS X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons &: member of the same patent family, corresponding document			

THIS PAGE BLANK (USPTO)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox

THIS PAGE BLANK (USPTO)